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The Development of an Edible Oil Flax Variety for Saskatchewan

Conducted by
G. Rowland

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Final Report

Submitted by:

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Executive Summary

Abstract. Linseed oil produced from flaxseed is a valuable industrial oil because of the high content of the fatty acid linolenic. If edible-oil forms of flax are to be developed then the content of linolenic acid in the oil must be greatly reduced. Edible-oil varieties of flax would help to further diversify cropping options and the products produced from Saskatchewan crops. To this end the flax variety McGregor was treated with a chemical that causes mutations. From this treated seed were grown plants, three of which contained mutations affecting the levels of linolenic acid in the seed. The one mutant, E1747, produced a line that contains only 2% linolenic acid and can thus be regarded as an edible-oil type. Mutant E67 has greatly elevated levels of palmitic acid that may be able to be developed as a replacement for palm oil. A high oleic acid mutant, E1929, will be useful in designing edible linseed oil tailored for specific markets.

Summary. In 1987 twenty thousand seeds of the flax variety McGregor were treated with the chemical ethyl methanesulphonate (EMS) with the purpose of producing mutants with lowered levels of the fatty acid linolenic in their seed. The high content of linolenic acid (53%) in linseed oil makes it valuable as a drying oil for the production of oil-based paints, linoleums and printers ink. However, linolenic acid is very unstable, it oxidizes rapidly and produces a rancid oil. For this reason it is not demanded for edible purposes. If the linolenic acid content of the oil could be substantially reduced then an edible linseed oil would be a result.

We have been very successful in reducing the linolenic acid levels of McGregor flax. One of the mutant lines uncovered has only 2% linolenic acid and can be regarded as an edible oil. E1747 has very high levels of linoleic acid (70%) and closely resembles sunflower oil. The mutant E67 has four times the level (28%) of palmitic acid found in McGregor. This mutation is simply passed on from generation to generation and may be useful as a replacement for palm oil in margarines. E1929 has elevated levels of oleic acid, which is found in abundance in olive oil. It is also simply passed on from generation to generation and should help in designing edible linseed oils for particular edible-oil uses and markets.

It appears that we will be able to produce linseed oils with a full range of oleic, linoleic and linolenic acid. This will mean that new human markets will open for linseed oil but also new industrial markets will be able to be investigated.

Background and Objectives

Vegetable oils are composed of fatty acids. Fatty acids determine the funcitonal characteristics of the oil and, therefore, how the oil will be used. These fatty acids are usually classified as saturated and unsaturated. Oils high in saturated fatty acids (palm oil) are generally solid at room temperature and oils high in unsaturated fatty acids (canola oil) are liquid. Unsaturated fatty acids have at least one chemical double bond. The more double bonds the less stable the fatty acid and the greater the proportion of these highly unsaturated fatty acids the more unstable the oil (i.e. the oil will become rancid). Canola

oil has a fatty acid profile of palmitic (4%), stearic (1%), oleic (60%), linoleic (25%) and linolenic (10%) and is reasonably stable. Linseed oil on the other hand has a fatty acid profile of Palmitic (6%), Stearic (4%), Oleic (18%), Linoleic (19%) and Linolenic (53%) and is quite unstable. Linseed oil is unstable because of the large amount of linolenic acid in the oil but it is also the fatty acid that makes it useful as a coating in the paint and linoleum industries. Linolenic acid contains three double bonds and is readily oxidized to form a film.

Several years ago Green (1986) using chemical mutagenesis produced a genotype of flax whose seed contained only 2% of linolenic acid. This discovery meant that linseed oil produced from this mutant could be used for edible-oil purposes rather than as an industrial oil for which linseed oil is traditionally used. The Crop Development Centre attempted to obtain this mutant line from CSIRO, Australia but the negotiations failed. Subsequently, CSIRO licensed the North American rights to this mutant to Biotechnica Canada of Calgary, a biotech firm with no experience in plant breeding and plant variety development.

The Crop Development Centre and CSP Foods both saw the tremendous advantages of an edible-oil flax to Saskatchewan. The Australian mutant was not likely to be available to us for the foreseeable future and there was doubt about the ability of Biotechnica to produce high yielding, well-adapted varieties of edible-oil flax for the prairies. Consequently, an application was made to the Agriculture Development Fund to begin the development of a variety of edible-oil flax adapted to Saskatchewan.

The objectives of the project, which cover only the initial stages of variety development, were:

1) The production of mutations in McGregor flax

2) The identification of mutations affecting the synthesis of linolenic acid

3) The placing of the mutant genes in a common genetic background in order to identify the correct combination that would lead to a low linolenic acid oil.

Experimental Methods

In May of 1987 20,000 seeds of the cultivar McGregor were treated with a 0.4% solution of ethyl methanesulphonate (EMS) after the method of Green and Marshall (1984). This seed was then planted at Kernen Crop Research Farm, University of Saskatchewan. The surviving 7,702 M_1 plants were harvested individually in the autumn.

The TBA (thiobarbaturic acid) test (Green and Marshall, 1984) was used to identify M_1 plants whose seed contained lowered levels of linolenic acid. Ten to fifteen half-seeds distal to the embryo axis from these plants were analyzed singly for their fatty acid content by gas chromatography. The embryo axis half was germinated in a petri dish on an agar-water medium. Half-seeds with reduced

linolenic acid levels had the germinated half transplanted to a pot and then grown in a growth chamber at 20°C day, 15°C night with a 16 h day.

Half-seeds from each of these M_2 plants were then analyzed on the GC and germinated as before. Once again seeds with reduced linolenic acid levels were grown. This procedure was repeated each generation.

Crosses were made among the three most interesting mutant lines tested. The F_1 generation of these crosses were reared in growth chambers and the progeny (individual seeds) analyzed as in the half-seed method described above.

Results and Discussion

A germination test of the EMS treated seed showed the germination to be over 80%. The fertility of most of the surviving plants was excellent in marked contrast to the reports of Green and Marshall (1984) and Nichterlein et al. (1988).

Over 70 lines (seed of M_1 plants) were identified by the TBA test as possibly having decreased linolenic acid levels. GC analysis of half-seeds from these lines identified seeds with reduced linolenic acid levels and the corresponding germinating half-seeds were then transplanted to soil in pots and raised to maturity in growth chambers. Three lines, E67, E1747 and E1929 became of immediate interest and have been raised through to the M_5 generation. All of these mutants have reduced levels of linolenic acid (Table 1) with E1747 having less than 2% linolenic acid which is very similar to the level of linolenic acid in the line described by Green (1986). However, unlike Green's material, the mutations, (if indeed there is more than one), were induced in a single M_1 seed. But this low linolenic acid level was not seen until seed from the M_3 generation was analyzed and not confirmed as being stable until the M_4 (Table 2). E67 has palmitic acid levels four times greater than that of McGregor or the other two mutant lines (Table 1). E1929 has oleic and linoleic acid levels double that of McGregor and we have just recovered some plants of 1929 whose seeds contained 50% oleic, 20% linoleic and 15% linolenic.

Table 1. Fatty acid composition, oleic desaturation ratio (ODR)+ and linoleic denaturation ratio (LDR)+ of the M_5 seed of three mutant lines and McGregor

Line	Fatty acid					ODR	LDR
	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)		
E67	27.8	2.8	11.3	6.6	44.0	5.0	7.1
E1747	7.0	3.2	15.8	71.8	1.9	5.1	0.03
E1929	7.1	2.7	34.7	30.1	25.2	1.8	0.9
McGregor	6.8	3.7	17.9	15.8	54.5	4.0	3.5

$$^{+}\text{ODR} = \frac{\% \text{ linoleic} + \% \text{ linolenic}}{\text{oleic}}$$

$$^{+}\text{LDR} = \frac{\% \text{ linolenic}}{\% \text{ linoleic}}$$

Each of the mutant lines has a different LDR from McGregor with E1747 and E1929 being lower and E67 higher. The ODR of E1929 is quite different from the rest. Thus each of the mutant lines is different from McGregor and from each other.

Table 2. Range of linolenic acid from the M_1 to the M_4 generation in E1747.

Generation and plant	Linolenic acid (%)
M_1 (1747)	40.5-53.2
M_2 (1747-5)	44.1-54.3
(1747-9)	19.1-44.5
M_3 (1747-9-1)	1.5-41.3
(1747-9-5)	1.8-30.3
(1747-9-7)	1.9-28.4
M_4 (1747-9-1-2)	2.3-45.2
(1747-9-1-4)	2.5-44.2
(1747-9-1-5)	1.4-3.1
M_5(1747-9-7-1-5)	2.0-3.0

There may be a question as to whether these mutant lines will be stable

when grown under field conditions. Preliminary testing carried out in the summer of 1989 indicated that these mutations in a homozygous state will be stable. Segregating M_3 lines of E67, E1747 and E1929 were planted for observation at the Kernen Farm. E67 produced seed with high levels of palmitic acid (Table 3), E1747 had some seed with 3% linolenic acid (Table 4) and all the seed of E1929 contained elevated levels of oleic and linoleic (Table 5).

Table 3. Fatty acid composition of two segregating M_3 lines of E67 grown at the Kernen Farm in 1989.

Seed	Palmitic	Stearic	Oleic	Linoleic	Linolenic
8-6-1	20.7	4.3	13.3	8.3	46.2
-2	19.3	4.7	11.9	8.1	50.5
-3	19.9	4.1	10.1	8.4	51.9
-4	21.1	3.3	13.7	9.6	46.0
-5	18.0	4.7	11.0	9.7	50.0
8-7-1	21.3	3.8	10.7	10.1	47.8
-2	12.9	4.0	10.2	12.5	56.2
-3	10.9	4.1	11.7	11.0	59.7
-4	19.2	3.7	9.8	8.4	53.3
-5	20.3	3.6	10.2	9.5	50.4

Table 4. Fatty acid composition of a segregating M_3 line of E1747 grown at the Kernen Farm in 1989.

Seed	Palmitic	Stearic	Oleic	Linoleic	Linolenic
9-3-1	5.5	4.5	17.3	50.5	21.8
-2	5.8	3.7	11.6	54.8	23.9
-3	6.2	4.2	11.8	35.8	41.6
-4	6.2	4.4	12.8	50.1	26.2
-5	5.8	4.5	16.3	69.7	3.4
-6	5.7	4.5	13.2	55.3	19.7
-7	6.1	4.6	15.7	70.1	3.1
-8	5.9	4.0	12.0	75.1	2.7
-9	5.7	4.4	15.0	55.0	19.6
-10	5.5	4.0	11.3	38.2	40.5

Table 5. Fatty acid composition of a segregating M_3 line of E1929 grown at the Kernen Farm in 1989.

Seed	Palmitic	Stearic	Oleic	Linoleic	Linolenic
10-3-1	5.7	4.5	37.0	29.9	22.3
-2	6.8	5.1	40.4	21.9	25.3
-3	6.5	4.6	33.8	23.1	31.5
-4	7.0	4.5	21.3	33.8	32.6
-5	6.4	5.6	35.3	32.5	19.4
-6	6.7	6.3	35.5	21.9	28.7
-7	6.1	6.2	35.6	32.9	18.2
-8	6.2	4.3	33.4	32.7	22.7
-9	6.5	6.4	34.6	30.0	17.9
-10	5.5	4.5	30.2	36.2	23.0

We have just identified an F_3 plant from the cross of E1747xE1929 that has 2% linolenic acid and has yellow seed. We thus have accomplished the final milestone laid out in our work plan. In addition, 1747 has been crossed to a number of other sources of the yellow seed character.

Crosses among the three mutant lines indicate that the mutations are simply inherited. When E1747 was crossed with E1929 the F_2 seed segregated in a 3:1 ratio of higher to 3% C18:3 (linolenic) (Fig. 1). Figures 2 and 3 illustrate the segregation for C18:3 with C18:2 (linoleic) and C18:1 (oleic) in this cross. In both instances there was a separation into high, medium and low C18:3 levels. This indicates that from this cross it should be possible to select for individuals with 3% C18:3 and equal amounts of approximately 40% C18:2 and 40% C18:1. It also should be possible to select for individuals that have 15-20% linolenic if industry requires such an oil.

The cross between E67, the high 16:0 (palmitic acid) mutant, and E1747, the low 18:3 mutant showed discrete segregation in the F_2 seed for high palmitic acid (Fig. 4). This along with Fig. 5 indicate the likely simple inheritance of the high palmitic acid character.

Conclusions and Impact

In one short year we have accomplished all the objectives set out in the work plan and indeed have moved substantially beyond what was proposed. We now can begin field evaluation of the low linolenic genotype one full year earlier than had been originally contemplated.

It also is going to be possible to produce and evaluate varieties of edible-oil flax that have different ratios of fatty acids. This will allow the

oil. Not only will it be possible to explore various edible-oil markets but we should also be able to design oils for specific markets.

The impact of this work for Saskatchewan is obviously very large. It will open numerous new markets for flaxseed and its oil. It will also increase the cropping options for Saskatchewan farmers but since most farmers already know how to produce flax, the usual problems associated with introducing a new crop will not arise.

References

Green, A.G. 1986. A mutant genotype of flax (Linum usitatissimum L.) containing very low levels of linolenic acid in its seed oil. Can. J. Plant Sci. 66: 499-503.

Green, A.G. and D.R. Marshall. 1984. Isolation of induced mutants in linseed (Linum usitatissimum) having reduced linolenic acid content. Euphytica 33: 321-328.

Nichterlein, K., R. Marquard and W. Friedt. 1988. Breeding for modified fatty acid composition by induced mutations in linseed (Linum usitatissimum L.). Plant Breed. 101: 190-199.

Papers and Talks

Rowland, G.G. and Bhatty, R.S. 1990. Ethyl methanesulphonate induced fatty acid mutations in flax. J. Am. Oil Chem. Soc. (in press).

Rowland, G.G. 1990. EMS induced mutations in McGregor flax. Proc. Flax Institute U.S. (in press).

Rowland, G.G. and Relf-Eckstein, J. 1990. Progress of the edible-oil flax program at the Crop Development Centre. Proc. Soils Crops Workshop (in press).

The latter two were conference presentations.

Statement of Expenditures

To be forwarded by the Research Accounts Office of the University of Saskatchewan.

Personnel Involved

Phyllis Mykota - April 15 to September 30, 1989 - full-time technician
Andrea Dorotich - May 1 to August 31, 1989 - full-time technician
Jo-Anne Relf-Eckstein - August 21, 1989 to present - full-time research assistant
Rosanne Beaule - September 25, 1989 to present - full-time technician
Doug Hassard - 5% of his time is spent on the project - technician
Geoff Galoway - 5% of his time is spent on the project - CSP Foods, technician
Dr. Gordon Rowland - 30% of my time is spent on the project - plant breeder
Dr. R. Bhatty - 5% of his time is spent on the project - chemist

Mykota, Dorotich, Relf-Eckstein and Beaule were paid from the project's funds.

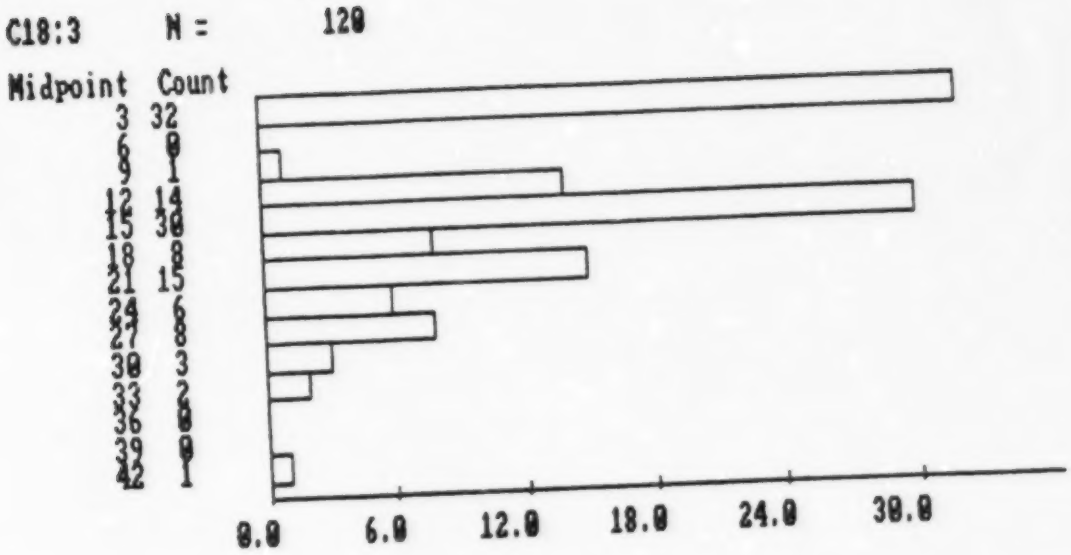

Figure 1. The frequency of various levels of linolenic acid (C18:3) found in F_2 seed of the cross E1747xE1929.

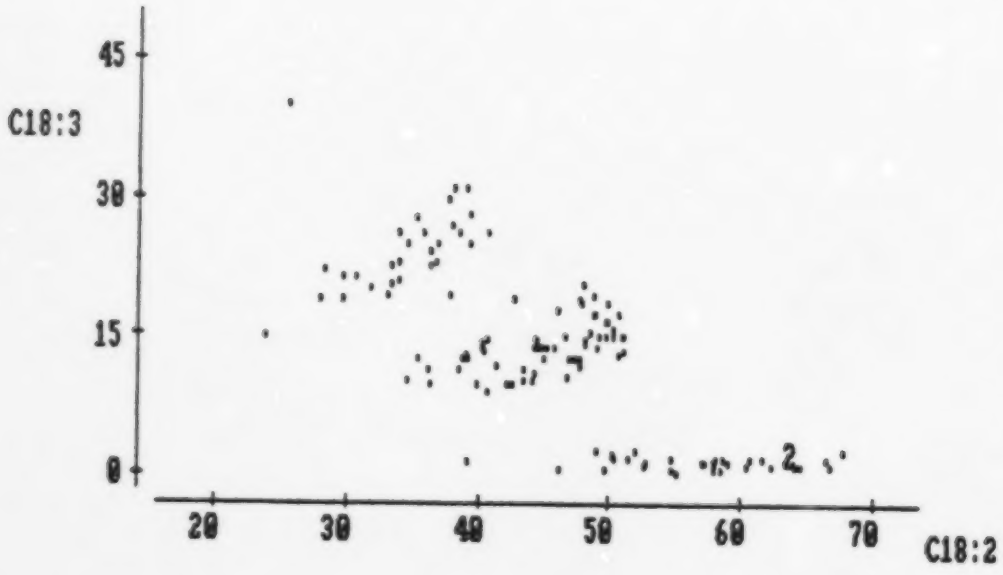

Figure 2. The level (%) of linolenic (C18:3) and linoleic (C18:2) acids found in individual F_2 seeds of the cross E1747xE1929.

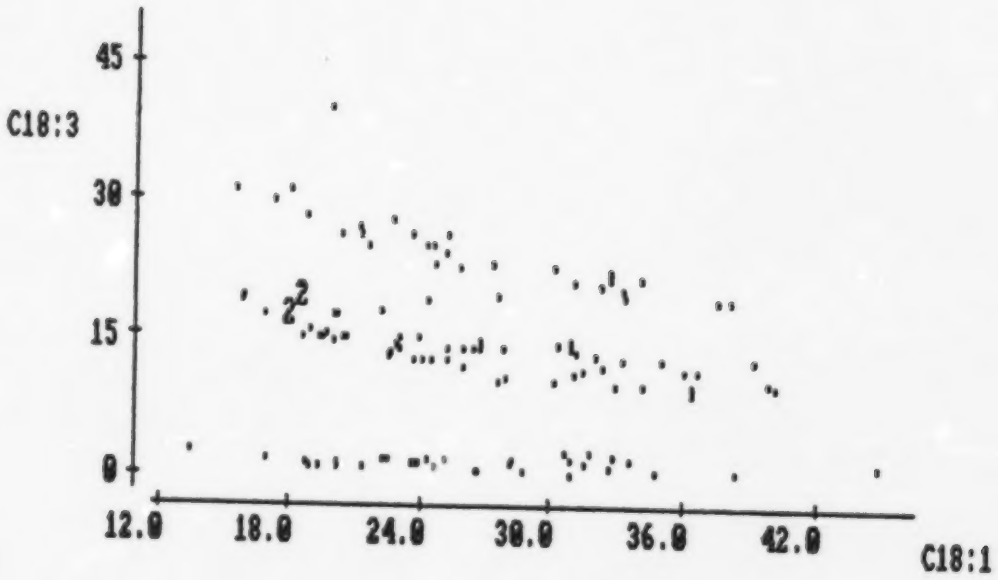

Figure 3. The level (%) of linolenic (C18:3) and oleic (C18:1) acids found in individual F_2 seeds of the cross E1747xE1929.

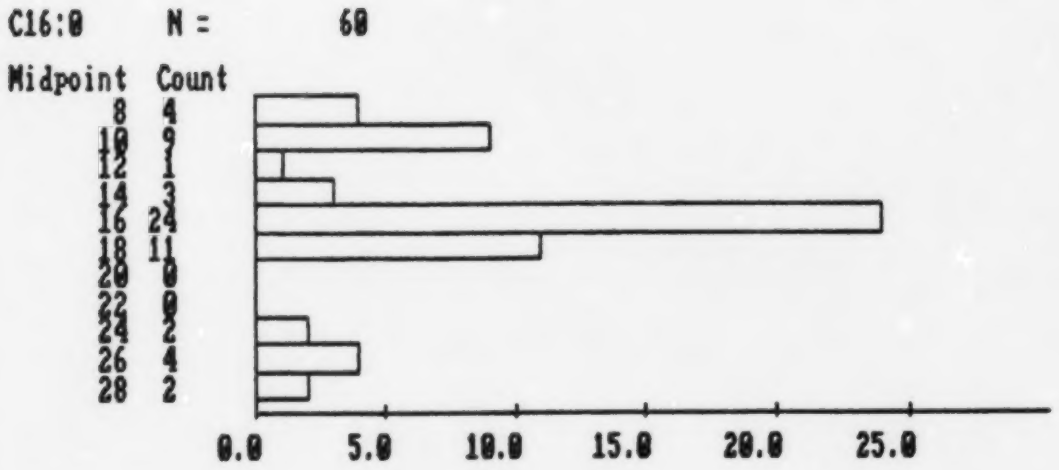

Figure 4. The frequency of various levels of palmitic acid (C16:0) found in F_2 seed of the cross E67xE1747.

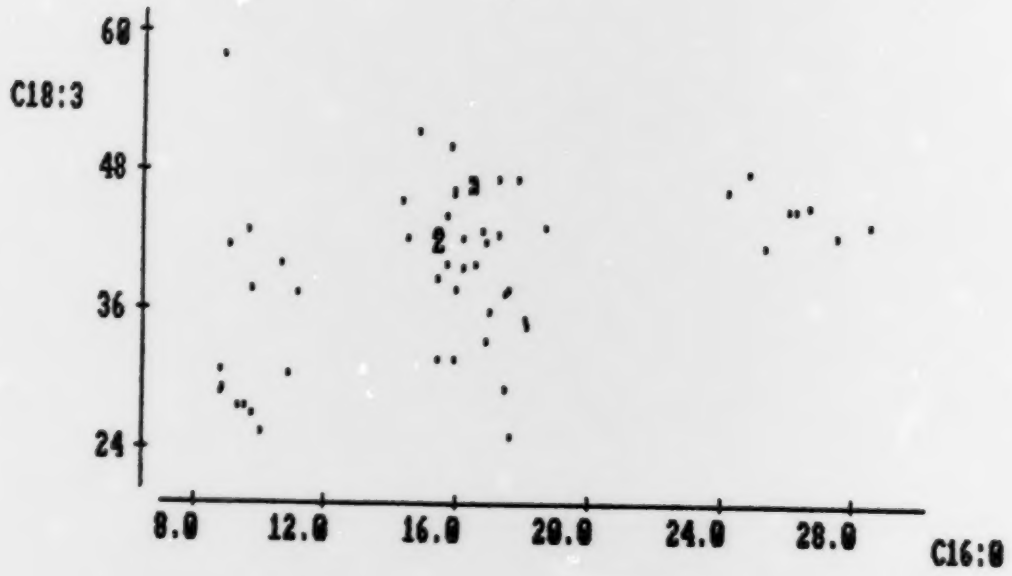

Figure 5. The level (%) of linolenic (C18:3) and palmitic (C16:0) acids found in individual F_2 seeds of the cross E67xE1747.